

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>5</sup> : G02B 21/34, G01N 1/30	A1	(11) International Publication Number: WO 94/23326 (43) International Publication Date: 13 October 1994 (13.10.94)
----------------------------------------------------------------------------------	----	-----------------------------------------------------------------------------------------------------------------------

(21) International Application Number: PCT/GB94/00671  
(22) International Filing Date: 30 March 1994 (30.03.94)  
(30) Priority Data:  
9306815.3 1 April 1993 (01.04.93) GB  
(71) Applicant (for all designated States except US): HYBAID LIMITED [GB/GB]; 111-113 Waldegrave Road, Teddington, Middlesex TW11 8LL (GB).  
(72) Inventors; and  
(75) Inventors/Applicants (for US only): SCOPES, Geoffrey, Eric [GB/GB]; 18 Ingram House, Park Road, Kingston, Surrey KT1 4BA (GB). MEYER, Edward [GB/GB]; 11 Shirley Avenue, Redhill, Surrey RH1 5AH (GB).  
(74) Agent: WILLIAMS, John, Francis; Williams, Powell & Associates, 34 Tavistock Street, London WC2E 7PB (GB).

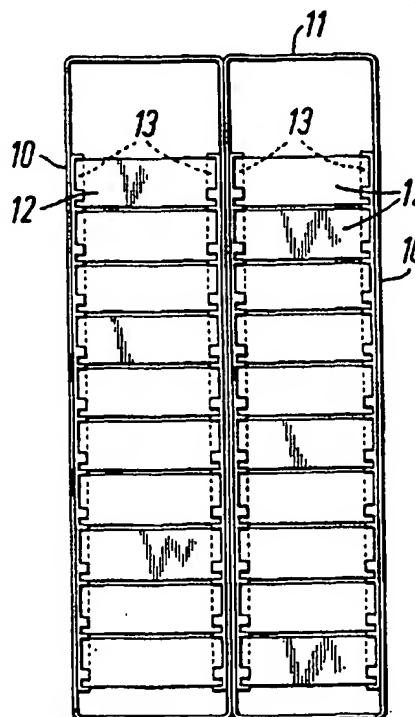
(81) Designated States: GB, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published  
With international search report.

(54) Title: MICROSCOPE SLIDE HOLDER

(57) Abstract

A microscope slide holder has a planar hollow frame (10) and series of clips (13) for retaining a number of microscope slides (12) in a generally flat plane. The holder is used for uniform processing, e.g. in-situ hybridisation, of the slides. A slim reaction chamber (35) can receive a slide holder for treatment using a minimum of reagent. A treatment apparatus receives a number of such chambers for heating and cooling.



BEST AVAILABLE COPY

AP

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

Microscope Slide Holder

This invention relates to a microscope slide holder, particularly a slide holder suitable for use in in-situ  
5 nucleic acid hybridisation.

Nucleic acid hybridisation is generally carried out with the target nucleic acid to be analysed immobilised in a solid support, particularly nylon membrane. In-situ  
10 hybridisation is a more sophisticated technique that enables the visualisation and the localisation of specific target DNA or RNA molecules in tissue sections, single cells or chromosome preparations. In this case, the support needs to be in the form of a  
15 microscope slide which can be examined in a conventional microscope. The success of any hybridisation process depends crucially upon control of temperature and time in the hybridisation cycle. Up to now, in-situ hybridisation has only been carried out using slides  
20 handled manually and individually between the various processes e.g. hybridisation, thermal cycling and slide washing. It has been very difficult to perform these processes accurately and reproducibly.

25 One key to achieving efficient in-situ hybridisation is the use of a slide holder which enables treatment of a plurality of slides at one time. Accordingly, the invention provides a microscope slide holder comprising a generally rectangular planar frame for receiving a  
30 plurality of microscope slides, having retaining means on the frame for individually retaining said slides in edge-to-edge relationship so that they cover space substantially in the plane of the frame.

2

Preferably, the retaining means comprise individual clips in which the slides are a sloppy fit and which permit movement of the slides perpendicular to the plane of the frame.

5

According to a further feature of the invention, there is provided a liquid reagent container for receiving said slide holder, which has a size closely matching that of a frame and into which said frame can be  
10 inserted through an open edge of the container.

Further, there is provided a processing unit having temperature control means and a plurality of slots for receiving a corresponding plurality of reagent  
15 containers as mentioned above.

Using such apparatus, 20 or more microscope slides can be put through identical treatment procedures, including temperature cycling, washing in water or other reagent  
20 when inserted in a reagent container, or heating in a suitable oven.

In order that the invention shall be clearly understood, one or more exemplary embodiments will now be described  
25 with reference to the accompanying drawings, in which:

Fig. 1 shows a microscope slide holder in plan view;

Fig. 2 shows a perspective view of the holder;

Fig. 3 shows three different forms of slide clip;

Fig. 4 shows a perspective view of a different slide  
30 holder;

Fig. 5 shows two views of a further form of clip;

Figs. 6 and 7 show different forms of clip employing bent wire;

Fig. 8 shows a processing unit; and

35 Fig. 9 shows liquid reagent containers for the slide holders.

In Fig. 1, two identical rectangular planar frames 10 are joined side-by-side. Each frame is dimensioned so as to receive ten standard microscope slides disposed in edge-to-edge relationship so that they cover substantially the whole space within the frame. A part of the frame is left empty to provide a form of handle 11 at the top. Each microscope slide 12 is supported at its ends by retaining means in the form of clips 13. The clips are designed so that the frame 10 can be laid horizontally or positioned vertically without the slides 12 falling out.

The same slide frame is shown in Fig. 2 in perspective view.

15

The precise nature of the clips 13 is illustrated in Fig. 3 which shows three different versions. The clip on the left has two projections 14 which are a press fit in holes 15 provided in the long sides 16 of the frames. In the middle and right-hand versions the clips engage the frames by virtue of channels 17 along their sides which are a snap fit onto the sides of the frames. In all three cases, the microscope slides 12 are shown to be a loose fit in the clips, such that if the frame is tilted slightly out of the vertical, the slides can tilt forwards (as illustrated) so that they are no longer coplanar but rather positioned in a series of parallel spaced-apart planes. In this position, it can be seen that each individual slide can be removed between two fingers of one hand. Conversely, the slides are easily placed in the clips by presenting them in a slightly tilted position and dropping them past the centre support 18 in each case. It can also be seen that if the frame is tilted backwards in the picture all the slides come to rest against the backs 20 of the clips and are in no danger of falling out if the frame is

positioned horizontally.

Fig. 4 shows a modified form of frame in which the individual clips 13 are formed as projections on a continuous moulded strip 21. Similar geometrical considerations apply as in Fig. 3.

Fig. 5 shows in greater detail a possible form of clip seen in perspective from both sides. For positioning the clips on the frame, two projections 24 are provided on one face, with a centre projection 25 having an enlarged sprung head, all of which can engage releasably in holes in the side of the frame 16. At the side of the clip which receives the microscope slide, a back surface 26 extends between L-shaped end retainers 27. A centre support 28 allows a slide inserted in the clip to tilt forwards without falling out.

Figs. 6 and 7 illustrate clips which are formed as a series of bent sections of a wire 30 extending the length of the frame. In Fig. 7, the clips only hold the microscope slides at one end and one of the slides 31 is shown in an angled position as it is inserted or extracted. In order to achieve this, the clip portion is swung in torsion around the vertical axis of the continuous wire 30.

Fig. 8 shows a processing unit having five slots 33 in its top surface. Each slot is designed to receive a complete microscope slide holder for treatment. Each holder is preferably first inserted in an envelope-like container 35, as illustrated in Fig. 9. Certain of the slots 33, for example numbers 1 and 2, may be provided with temperature control means such as a water bath or sources of radiant heat; others of the slots may accept slide holders and/or reagent containers for maintenance

at ambient temperature.

Fig. 9 shows a number of liquid reagent containers 35, one containing a microscope slide holder as previously described. The handle portion 11 of the frame projects at the top of the container. The container 35 is a relatively tight fit around the slide holder and the holder is inserted through one open edge 36 of the container. It can be seen that this arrangement minimises the quantity of liquid reagent which has to be placed in the container in order to treat the microscope slides. Moreover, since the liquid quantity is small, it can rapidly and accurately be heated or cooled. Each container has a widened top 37 to simplify filling and emptying.

The microscope slide holder described is advantageous in that a plurality of slides can be uniformly treated; each slide can easily be inserted and removed from the holder; and the holder can be positioned vertically to be placed in a reagent container or positioned horizontally for placing on a heater plate for temperature treatment. In particular, when placed horizontally, the slides still have a freedom within the clips to move perpendicular to their own planes. This enables a holder full of slides to be placed on a horizontal heated surface which is slightly smaller than the internal dimensions of the frame and the clips. When this is done, the slides necessarily come to lie flat in contact with the heated plate, while the frame can drop slightly and indeed comes to exert a slight downward pressure on all the slides. By contrast, if all the slides are rigidly held in the frame, any slide not exactly coplanar with the majority would not lie in face-to-face contact with the heater plate.

6

The apparatus described above is particularly adapted to the process of in-situ hybridisation and provides a means of carrying out all the processes necessary in uniform fashion for a relatively large number of  
5 samples.

10

15

20

25

30

35

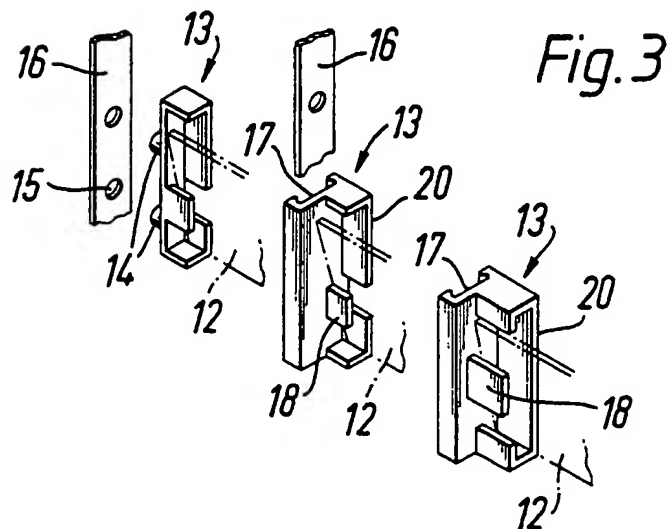
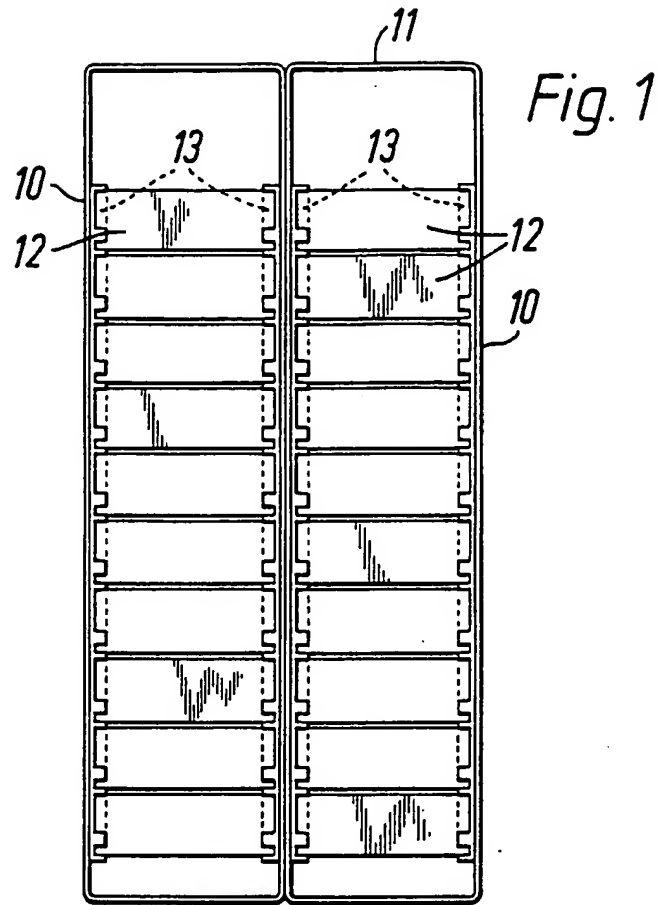


CLAIMS

1. A microscope slide holder comprising a generally rectangular planar frame (10) for receiving a plurality of microscope slides (12), having retaining means (13) on the frame for individually retaining said slides (12) in edge-to-edge relationship so that they cover space substantially in the plane of the frame.
2. A microscope slide holder as claimed in claim 1 wherein said retaining means (13) are arranged along at least one side (16) of the frame.
3. A microscope slide holder as claimed in claim 1 or 2 wherein the retaining means (13) are formed as a series of inward projections (18, 20, 26, 27, 28) on the frame (10).
4. A microscope slide holder as claimed in claim 3 wherein the retaining means for one or more slide(s) is a discrete unit (13, 21) having said projections, which unit is clipped (14, 15, 17) onto said frame.
5. A microscope slide holder as claimed in claim 1 or 2 wherein the retaining means are formed as clips bent at intervals in a continuous metal member (30) which extends parallel along a side of the frame.
6. A microscope slide holder as claimed in any preceding claim which includes a handling portion (11) at one end of the frame which is not provided with retaining means for slides.

7. A microscope slide holder as claimed in any preceding claim wherein the retaining means (13) retains each slide without gripping it.
- 5 8. A microscope slide holder as claimed in any preceding claim wherein the holder retains the sides (12) when the frame is disposed in both a generally vertical and a generally horizontal plane.
- 10 9. A microscope slide holder as claimed in claim 7 wherein slides can be tilted about an edge to insert or remove them in or from the retaining means.
- 10 10. A reaction apparatus comprising a microscope slide  
15 holder as claimed in any preceding claim, and a chamber (35) for receiving liquid reagent which chamber has internal dimensions very slightly larger than the external dimensions of the slide holder.
- 20 11. A reaction apparatus as claimed in claim 10 wherein the slide holder is fully accommodated within the chamber with only a handling portion (11) accessible outside it.
- 25 12. A reaction apparatus as claimed in claim 10 or 11 wherein the chamber is open along one edge (36).
13. A reaction apparatus as claimed in claim 12 wherein the said open edge (36) is splayed to form a  
30 pouring lip (37).
14. A treatment apparatus comprising a housing and heating and cooling means with control means on or in the housing, wherein the housing has one or more top  
35 apertures (33) which receive one or more reaction apparatuses as claimed in any of claims 10 to 13.

1/4



2/4

Fig. 2

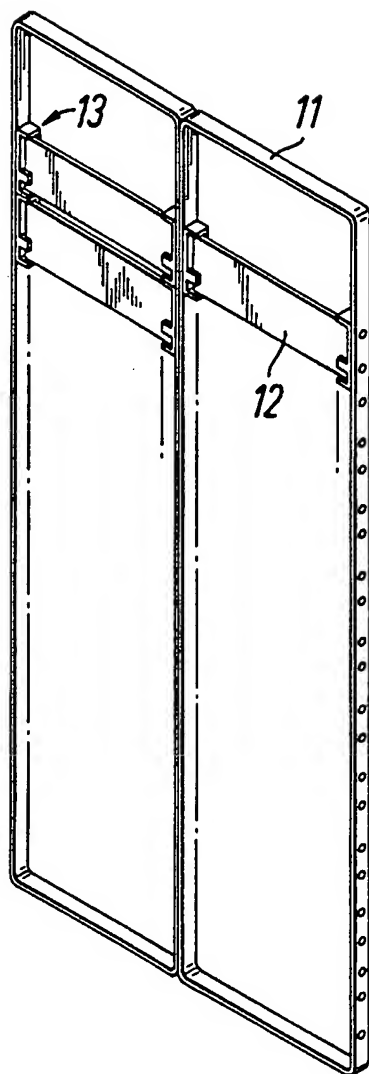
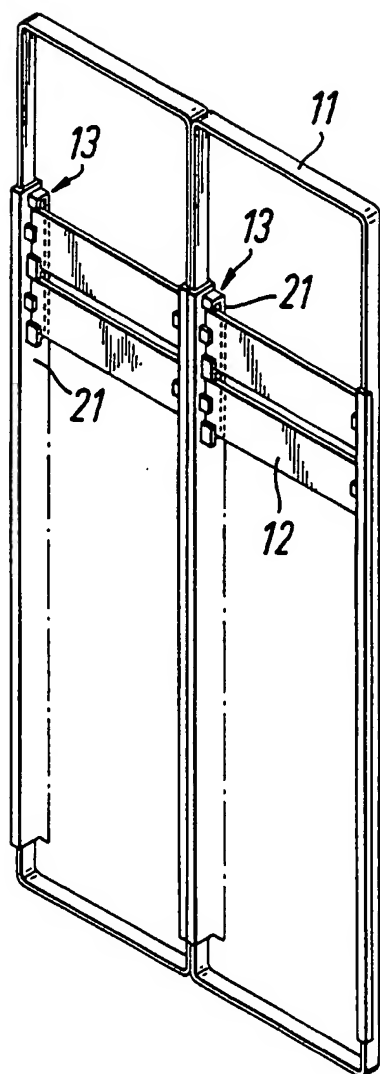
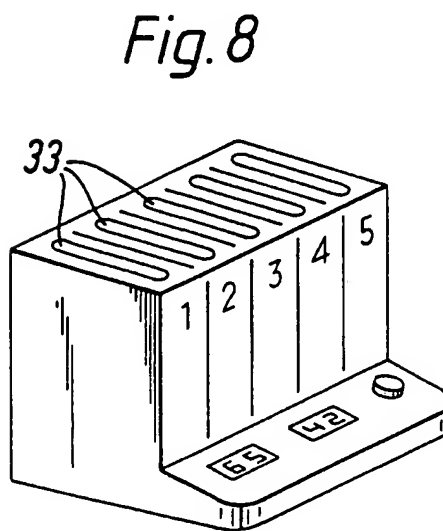
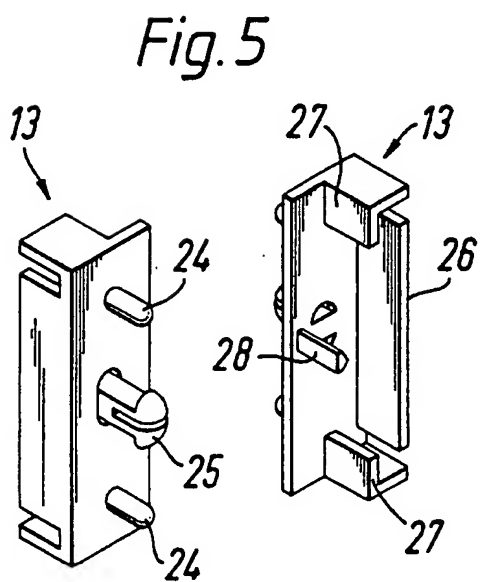
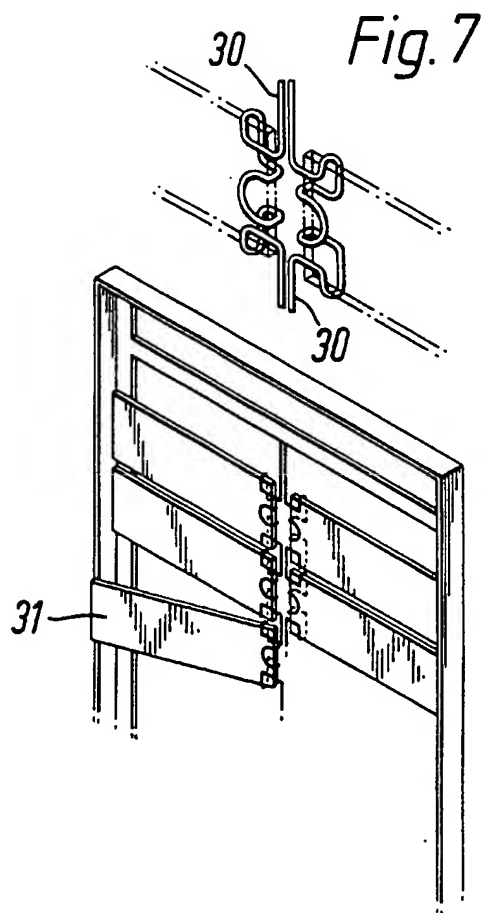
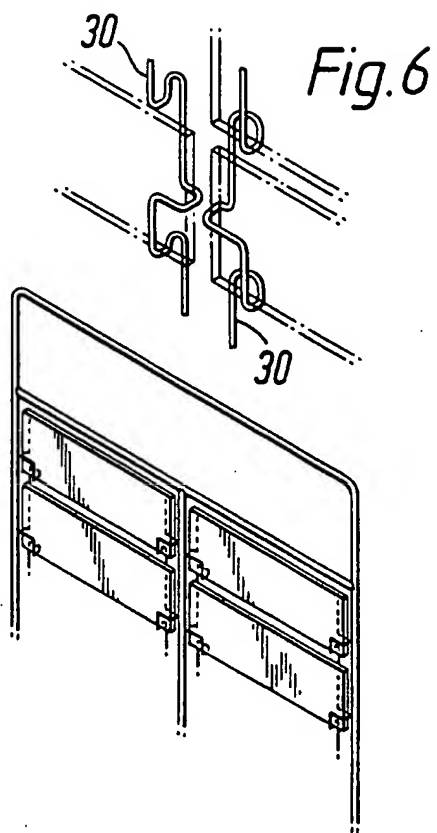


Fig. 4

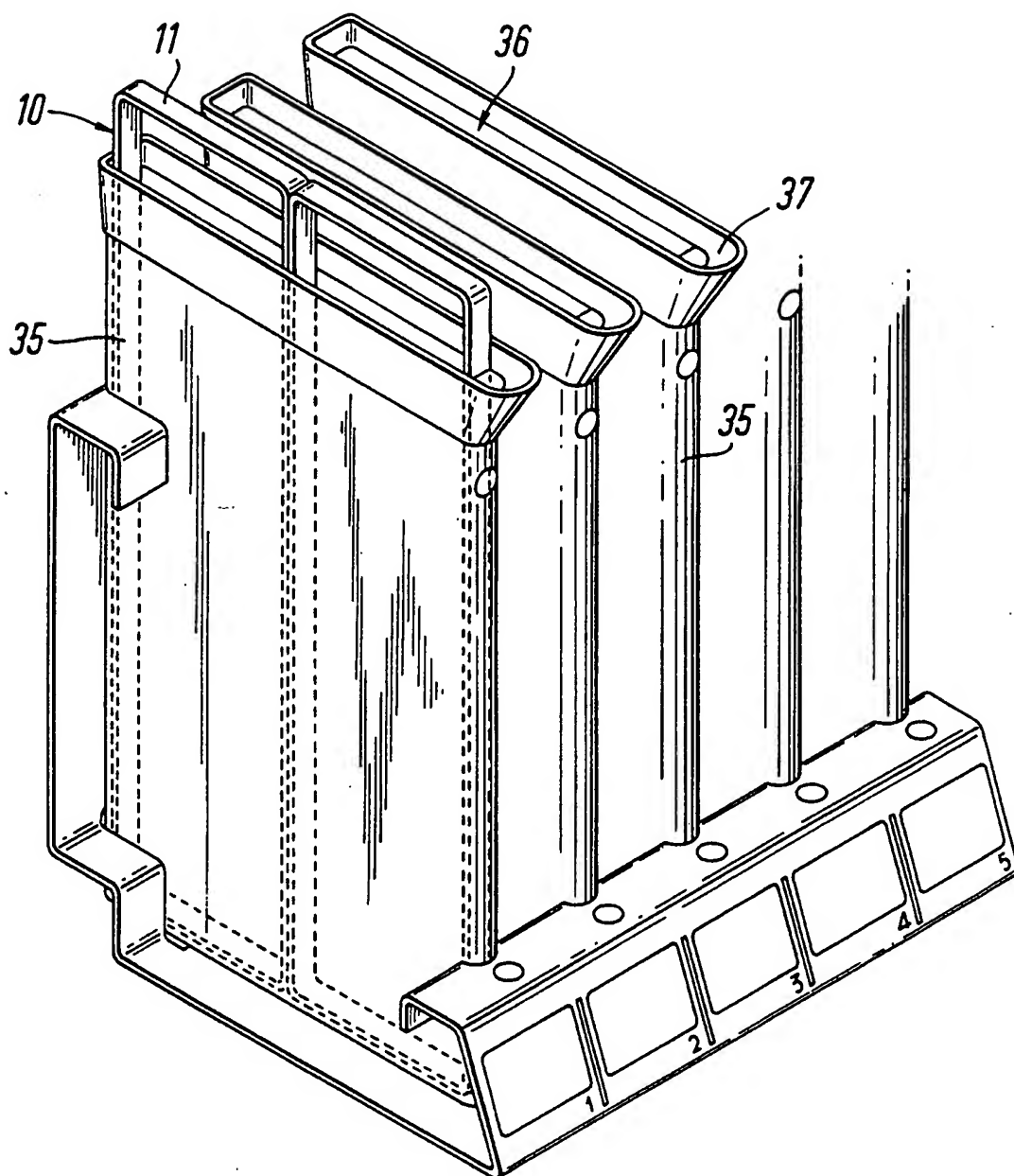


3/4



4/4

Fig. 9



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 94/00671

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 G02B21/34 G01N1/30

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 5 G02B G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FR,A,2 508 184 (SMITHKLINE BECKMAN) 24 December 1982 see page 8, line 22 - page 10, line 30; figures 4,5,12 ---	1-3,6-8, 10-14
Y	US,A,3 667 088 (F.D.KURTZ) 6 June 1972 see the whole document ---	1,2,8
Y	EP,A,0 415 400 (G.MENZEL GLASBEARBEITUNGSWERK) 6 March 1991 see column 3 - column 6; figures 1-18 ---	1-3,7,8
Y	US,A,2 864 105 (J.ROSE) 16 December 1958 see the whole document ---	6
Y	GB,A,219 119 (F.W.JACKSON) 9 November 1983 see page 4; figures 5-16 ---	10-13
	--- -/--	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "B" earlier document but published on or after the international filing date
- "I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

9 June 1994

Date of mailing of the international search report

15.06.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Malic, K

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 94/00671

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A,3 132 744 (B.C.SCHARF) 12 May 1964 see column 3; figures 1-4 ---	10-13
Y	WO,A,92 21953 (MEDITE GESELLSCHAFT FÜR MEDIZINTECHNIK MBH) 10 December 1992 see abstract; claim 42; figures 1-12 -----	14



**INTERNATIONAL SEARCH REPORT**

Information on patent family members

Intern. Application No

PCT/GB 94/00671

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A-2508184	24-12-82	US-A- 4453807	12-06-84
		CA-A- 1179534	18-12-84
		DE-A, C 3222594	05-01-83
		GB-A, B 2107664	05-05-83
		JP-A- 58002817	08-01-83
		US-A- 4501495	26-02-85
-----			
US-A-3667088	06-06-72	NONE	
-----			
EP-A-0415400	06-03-91	US-A- 5044500	03-09-91
		CA-A- 2023604	02-03-91
		DE-U- 9012467	22-11-90
-----			
US-A-2864105		NONE	
-----			
GB-A-219119		NONE	
-----			
US-A-3132744		NONE	
-----			
WO-A-9221953	10-12-92	DE-A- 4117833	03-12-92
		EP-A- 0586494	16-03-94
-----			